GEORGIA TECH LIBRARY

10:00:26 OCA PAD INITIATION - PROJECT HEADER INFORMATION 08/18/87

Project # G-33-G12 MOD # REV # 0
Contract # 2 R01 GM18894-17 OCA file # Status A
Contract entity ~~GTRC~~ GIT Prime contract #
PDPI YU N (DR.
SSN - - Unit CHEM Phone ( ) -
Project unit CHEM Unit code 02.010.136
Sponsor/Division DHHS/PHS/NIH / NATL INSTITUTE OF HEALTH
Sponsor#/division # 108 / 001
Type of document GRANT
Award period: from 87 / 09 / 01 to 88 / 08 / 31 (perf) 88 / 11 / 30 (rpts)

Sponsor amount	New this change	Total to date
Contract value	$ 177006	177006
Funded	$ 177006	177006

Cost sharing # Cost sharing $
Does subcontracting plan apply? (Y/N) N
Title -
LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

CTR project # Q5169-2A0 CTR cost sharing #

Are there existing subprojects? (Y/N) N
Is this a subproject? (Y/N) N Main project #
Continuation of project # G-33-G11/Q5169-1A0 Type of research RES

Coproject director name

SSN - - Unit

Coproject director name

SSN - - Unit

PROJECT ADMINISTRATION DATA

Administrative data OCA contact E. FAITH GLEASON PAD CO EFG 894-4820
Sponsor technical contact
DR. JAMES CASSATT
(301) 496 - 7309
NAT INST GENERAL MEDICAL SCIENCES
BETHESDA, MD 20892

Sponsor issuing office
JEFF CAROW, JANE JULIA, ANN CALURE
(301) 496 - 7166
OFFICE OF ASSOCIATE DIRECTOR PROGRAM
ACTIVITIES, NIGMS
BETHESDA, MD 20892

Security class (U,C,S,TS)
Defense priority rating N/A
ONR resident rep. is ACO (Y/N) N
_____ supplemental sheet
Equipment title vests with Sponsor _ GIT X Comment follows -
INTERNAL PRIOR APPROVAL REQUIRED IF NOT INCLUDED IN AWARD BUDGET

Admin comments -
17TH YEAR OF CONTINUING GRANT APPROVED FOR 21 YEARS.

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 9/16/88

Project No. G-33-G12/243051692A0 School/Lab Chemistry

Includes Subproject No.(s) N/A

Project Director(s) Dr. Nai-Ten Yu GTRC/GIT

Sponsor DHHS/PHS/NIH

Title Laser-Excited Raman Spectroscopy of Biopolymers

Effective Completion Date: 8/31/88 (Performance) N/A (Reports)

Grant/Contract Closeout Actions Remaining:

- [ ] None
- [X] Final Invoice or Copy of Last Invoice Serving as Final
- [ ] Release and Assignment
- [ ] Final Report of Inventions and/or Subcontract:
  Patent and Subcontract Questionnaire sent to Project Director [ ]
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other _____

Continues Project No. G-33-G11 Continued by Project No. G-33-G13

COPIES TO:

Project Director
Research Administrative Network
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Reports Coordinator (OCA)
Program Administration Division
Contract Support Division (2)

Facilities Management
Library
GTRC
Project File
Other _____

1. The Plans for the Next Year of Support : We will carry out experiments with the following specific aims---(a) To determine the effects of distal H-bonding on ligand vibrations in a series of synthetic porphyrins with functional groups ($-CH_2OH$, $-COOH$, $-CONH_2$, $-CONHNH_2$) near the metal center, capable of forming H-bonds of varying strength; (b) To determine the effects of selective distal amino acid replacements on ligand bonding interactions in the β-chain of human Hb (comparison of results between - and --chain mutations); (c) To obtain and analyze the excitation profiles of deoxy Fe(II)adamantane porphyrin-6,6-cyclophane with N-methyl imidazole as an axial base; (d) To initiate large preparation of Dd Hb from liver fluke and to search for the origin of extremely high ligand affinity; (e) To determine the effects of NO ligand distortion on the Mn-N-O bending mode RR intensity.

2. Concise Description of the Studies Conducted during the Current Year :
   (a) Raman Evidence for Non-Coupling of Individual 2- and 4- Vinyl Vibrational Modes in Monomeric Cyano-Met Hb------We have studied the RR spectra of monomeric insect cyano-met hemoglobins reconstituted with protoheme-IX selectively deuterated at the 4-vinyl as well as the 2,4-vinyls, pemptoheme, isopemtoheme and symmetric hemes (protoheme-III and -XIII). Evidence has been obtained that the highly localized vinyl C=C stretching vibrations at the 2 and 4 positions of the heme in these cyano-met CTT Hbs are non-coupled and inequivalent: the 1631 cm^{-1} and the 1622 cm^{-1} lines have been assigned to 2-vinyl and 4-vinyl, respectively. In the low-frequency region, the 412 cm^{-1} line is primarily derived from 2-vinyl, whereas the 591 cm^{-1} line comes from the 4-vinyl.
   (b) Heme-Rotational Disorder in Monomeric Allosteric Cyano-Met Insect Hemoglobins Detected by Resonance Raman------In deutero-IX and meso-IX reconstituted CTTs, we have identified two Fe-C-N bending vibrations at 401 cm^{-1} and 422 cm^{-1} at pH 9.5) for ^{57}Fe deutero-IX CTT IV ligated with $^{13}C^{15}N$, which are attributed to the two heme-rotational components. One Fe-C-N bending mode at 422 cm^{-1} shows a pH-induced shifts to 424 cm^{-1}(pH 5.5) indicating the t-> r conformational transition, whereas the other bending mode is pH-insensitive, representing a non-allosteric component.
   (c) The Bonding Interactions between Mn and NO ligand in Monomeric Nitrosyl Manganese CTT IV------We have identified the Mn(II)-NO stretching mode at 628 cm^{-1}, the Mn(II)-N-O bending mode at 574 cm^{-1} and the N-O stretching mode at 1735 cm^{-1}. The results suggest a strong Mn(II)-NO bond and a weak N-O bond. In comparison, in carbonmonoxy CTT IV the Fe(II)-CO stretching mode at 500 cm^{-1} and the C-O stretching mode at 1950 cm^{-1}, indicating a weaker Fe-CO bond and a stronger C-O bond.
   (d) Sterically Hindered Cyano-Met "Strapped" Hemes------We have determined the iron-carbon bond stretching vibrations, ν(Fe-CN), in sterically hindered and unhindered heme Fe(III)-CN^- complexes. Our study reveals that the ν(Fe-CN) frequency decreases as the chain length is decreased, in contrast with the

CO complexes, where the ν(Fe-CO) frequency increases as the chain length is decreased. We have also studied the cyanide complexes with three different bases (pyridine, N-methylimidazole and 1,2-dimethylimidazole), and found that the trans-effect of cyanide complex is different from that of CO complexes. (See the attached reprint : Biophys. J. 52, 801-805(1987)).

(e) E.coli -synthesized Human Hb Mutants----In collaboration with Drs. Nagai and Shih, we have studied the RR spectra of mutant Hbs produced in E. coli having Val(67β) E11 replaced by Ala, Met, Leu or Ile and His(58β)E7 by Gln, Val or Gly. The resonance Raman results were compared with those from equilibrium and kinetic studi of ligand binding. It is concluded that the steric hindrance of ligand binding by the E11 residue and the polarity of the E7 residue in the β-subunit are critical fo fine-tuning ligand affinity. See attached reprint: Nature, 329, 858-860(1987).

3. Not Applicable.

4. Not Applicable.

DO NOT TYPE IN THIS SPACE—BINDING MARGIN

5. Publications:

(a) Gersonde, K., Yu, N.-T., Kerr, E.A., Smith, K.M. and Parish, D.W.(1987) "Heme-rotational Disorder in Monomeric Allosteric Cyano-Met Insect Hemoglobins Monitored by Resonance Raman Spectroscopy" J. Mol. Biol. 194, 545-556.

(b) Lin, S.-H., Yu, N.-T. and Gersonde, K.(1988)"Resonance Raman Evidence for an Unusually Strong Exogenous Ligand-Metal Bond in a Monomeric Nitrosyl Manganese Hemoglobin" FEBS Lett., 229, 367-371.

(c) Tanaka, T., Yu, N.-T. and Chang, C.K.(1987) "Resonance Raman Studies of Sterically Hindered Cyanomet "Strapped Hemes" Biophys. J., 52, 801-805.

(d) Tsubaki, M., Hori, H., Hotta, T., Hiwatashi, A., Ichikawa, Y. and Yu, N.T.(1987) "Influence of Heme-Surrounding Amino Acid Residues on the Manganese(V)-Nitrido Bond in Manganese-Substituted Hemoproteins: Resonance Raman Evidence for Porphyrin Core Expansion and Reduction of the Manganese(V)-Nitrido Stretching Force Constant" Biochemistry, 26, 4980-4986.

(e) Nagai, K., Luisi, B., Shih, D., Miyazaki, G., Imai, K., Poyart, C., De Young, A., Kwiatkowsky, L., Noble, R.W., Lin, S.-H. and Yu, N.-T.(1987) "Distal Residues in the Oxygen Binding Site of Hemoglobin Studied by Protein Engineering" Nature, 329, 858-860.

(f) Gersonde, K., Yu, N.-T., Lin, S.-H., Smith, K.K. and Parish, D.W.(1988) "Resonance Raman Assignment and Evidence for Non-Coupling of Individual 2- and 4-Vinyl Vibrational Modes in a Monomeric Cyano-Met Hemoglobin" J. Mol. Biol. (submitted).

DO NOT TYPE IN THIS SPACE—BINDING MARGIN

CHECKLIST

INDIRECT COST CALCULATION

GRANT NUMBER

GM 3894-18

Check the appropriate boxes and provide the information requested. Make this page the last page of the signed original of the application. ***Do not attach copies of this page to the duplicated copies of the application.***

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of for-profit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will *not* be paid on foreign grants, construction grants, grants to Federal organizations and grants to individuals, and usually not on conference grants. Follow any additional instructions provided for Research Career Development Awards, Institutional National Research Service Awards, and specialized grant applications.

☐ DHHS Agreement Dated: _____

☐ No Indirect Costs Requested

☒ No DHHS Agreement, but rates established with ONR

DATE 7/1/87

***CALCULATION**

Enter proposed budget period:

Amount of Base $ 106,497 × Rate Applied 60.0* % = Indirect Costs $ 63,898

Add to total direct costs from page 2 and enter new total on FACE PAGE, Item 10b

*Check appropriate box(es):

☐ Salary and wage base ☒ Modified total direct costs base ☐ Other base *(Explain below.)*

☐ Off-site, other special rate, or more than one rate involved *(Explain below.)*

Explanation *(Attach separate sheet, if necessary.)*

*** Use Site 02-Other Direct Cost**